EXPLORATION OF THE STRATOSPHERE FOR VIABLE MICROORGANISMS

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ABSTRACT

Two balloon-borne flights to 19.8 km and 27.1 km were conducted in 1962 to sample air volumetrically through filters and to determine the existence and identity of viable microorganisms in the stratosphere. Although a variety of bacteria, yeasts, and molds were recovered from filters exposed at high altitudes, malfunctions of the mechanical equipment and sterile controls limit the conclusions that can be drawn from these experiments. The feasibility of this type of exploration and the precautions which must be taken are discussed.

INTRODUCTION

The study of airborne microorganisms has occupied the attention of microbiologists since the classical work of Pasteur over one hundred years ago. In particular, experiments designed to measure microbial distribution in the air, the factors which influence airborne dissemination, and the phenomena associated with survival of microbial aerosols have been of interest to such diverse scientific disciplines as meteorology, public health, particle technology, ventilation engineering, and the plant sciences, as well as to general microbiology. Attempts with varying degrees of success have been made by workers in many countries to develop suitable sampling apparatus, and to evaluate quantitatively and qualitatively the microbial aeroflora of extramural and intramural environments (AAAS, 1942; Gregory, 1962).

A specialized and relatively unexplored area in the general field of aeromicrobiology is research to determine the existence and identity of viable microorganisms at high altitudes, particularly above the tropopause. Increased information about stratospheric biology would have several interesting theoretical and practical implications. It would expand biological knowledge about an environment which at one time was distant and remote, but which is today of intimate concern to aerospace workers. Knowledge about stratospheric organisms would help elucidate certain basic problems about the existence and maintenance of viability in a hostile environment. It could increase our understanding of protective mechanisms employed by lower life forms during dormancy. Furthermore, research in stratospheric biology would supplement the body of data that has been accumulated about the biology of lower air strata and could ultimately provide a rational hypothesis about generation and dissemination of microorganisms on earth.

Meteorologists and plant scientists are interested in stratospheric microorganisms because of their possible use as tracers of global air movements and the concomitant long-range long-time dispersal of biological material. Exobiologists hope that stratospheric biological probes will elucidate aspects of the Panspermia hypothesis. At the very least, knowledge gained from such studies will help us to plan experiments for detection of living entities in the "atmospheres" of other planets. From a practical point of view, stratospheric microbiology studies will provide some foreknowledge about potential contamination of space vehicles launched from the earth.

PREVIOUS STUDIES

Despite the great interest in this field of study, and the many attempts to investigate the microbiological distribution in air at altitudes above ground level (Table I) no reliable information is available about microorganisms in air strata above 7000 m. Even the extensive investigations at lower altitudes were handicapped by one or more of the following limitations: (page 6)

Table I. Selected Investigations in High Altitude Microbiology*

Investigator or Team	Dates	Maximum Altitude Above Local Ground Level (M)	Vehicle	Sampling Technique	Geographical Location
Blackley	1873	300	Kite	Sticky Slides	Manchester
Miquel	1883	?	Tower	Volumetric Filtration	Paris
Carnelley	1887	?	Building	Volumetric Impaction on Nutrient Surface	London
Cristiani	1893	1700	Balloon	Volumetric Liquid Impinger	Geneva
Harz	1904	2300	Balloon	Volumetric Filtration	Bavaria
Flemming	1908	4100	Balloon	Volumetric Filtration	Berlin
Hahn	1909	3500	Balloon	Volumetric Filtration	S. Germany
U of Minnesota (Stackman et al.)	1921	5000	Aeroplane	Sticky Slides	Central USA
Scheppergrell	1923-25	5800	Aeroplane	Sticky Slides	Central USA
Mischustin	1923	2000	Aeroplane	Ram Impaction on Nutrient Plates	Moscow

*Information on works by authors listed here can be found in the bibliographies of books referenced at the conclusion of this paper.

Table I. Selected Investigation in High Altitude Microbiology (Continued)

Investigator or Team	Dates	Maximum Altitude Above Local Ground Level (M)	Vehicle	Sampling Technique	Geographical Location
Dominion Rust Labs (Craigie et al.)	1928-31	4260	Aeroplane	Sticky Slides	Manitoba
Dillon Weston	1929	3000	Aeroplane	Nutrient Plates	Cambridge
Brown	1930	2200	Aeroplane	Nutrient Plates Oiled Slides	SW USA
Cotter	1931	3000	Aeroplane	Oiled Slides	Lake Michigan
Hubert	1932	800	Balloon	Spore Trap	Germany
Durham	1932-40	4000	Aeroplane	Sticky Slides	Cont. USA
U. S. D. A. (Meier et al.)	1932-38	5500	Aeroplane	Nutrient Plates Sticky Slides	N. Atlantic Carribean Cont. USA
Proctor & Parker	1932-42	6000	Aeroplane	Lens Paper Filters	Cont. USA
MacQuiddy	1935	2100	Aeroplane	Nutrient Plates Slides	Nebraska
MacLachlan	1935	600	Aeroplane	Nutrient Plates	Massachusetts
Walker	1935	9000	Aeroplane	Nutrient Plates	SW USA
Mehta	1936-38	3000	Balloon	Sticky Slides	India

Table I. Selected Investigations in High Altitude Microbiology (Continued)

Investigator or Team	Dates	Maximum Altitude Above Local Ground Level (M)	Vehicle	Sampling Technique	Geographical Location
Rogers & Meier	1936	21000	Balloon Ascent Parachute Descent	Ram Impaction Glycerined Surface	Dakotas
Rempe	1937	2000	Aeroplane	Sticky Slides	Germany
Van Overeem	1937	2000	Aeroplane	Filtration	Netherlands
Skrzynska	1938-39	6000	Aeroplane	Oiled Lens Paper Filter	Poland
Wolf	1943	3250	Aeroplane	Nutrient Plates	Tennessee
McGill University (Polunin et al.)	1947-51	6770	Aeroplane	Siliconed Slides Electrostatic Precipitator Filters	Arctic N. Canada N. Atlantic
Heise	1948	3000	Aeroplane	Sticky Slides	Milwaukee
Krishko et al.	1957-62	7000	Aeroplane	?	USSR

1) Lack of efficient sampling devices: attempts to sample microorganisms with such devices as oiled lens-paper filters, sticky slides, and nutrient agar dishes exposed from moving aircraft leave much to be desired. Whereas large particles ($>10\mu$) such as pollen grains and certain fungus spores might be efficiently impacted out of an aerosol, bacterial cells and spores ($<5\mu$) are considerably more difficult to collect by these techniques.

2) Lack of large volume samples and volumetric measurement devices: When sampling the atmosphere where the total microbial population is low ($<5/m^3$), and subsequently returning the sample for analysis to ground levels and laboratory environments where the contamination level is higher by one or more orders of magnitude, a very large sample must be taken to increase the "signal" relative to the "noise". Most of the studies reported to date did not sample sufficiently large air volumes to make reliable inferences about microbial concentrations in the atmosphere. Furthermore, many of the previous studies did not adequately measure the air volume sampled, and should be considered essentially qualitative.

3) Inability to sample quantitatively at high altitudes: The ceiling tolerances of manned aircraft built before World War II, and the inadequacies of automatic sampling devices suitable for stratospheric balloon flights limited most aerobiological explorations to the troposphere.

4) Lack of adequate controls and sterility precautions: Many of the previous reports on upper-air sampling may be criticized because of inadequate sterility precautions during assembly, handling, and analysis of the sampling apparatus.

The most significant effort to date in the field of stratospheric microbiology was the National Geographic Society experiment from the balloon balloon Explorer #2 (Rogers & Meier, 1936). After ascending to 22,000 m, a sterile tube sampler was released to descend by parachute and to sample a profile extending from 21,000 m to 11,000 m, where a barometric device closed the inlet parts with cotton filters. Sampling was carried out by directing the internal air stream against glycerine-coated walls. The authors assumed that they sampled a column of air 10.5 km x 8.7 cm or a total volume of approximately 72 m^3. Upon laboratory examination, they cultured 10 microbial colonies, leading to a calculation of 0.14 organisms/m^3. Despite the obvious criticism which can be leveled against this experiment in retrospect, it is important to note that this work was carried out nearly 30 years ago and that it was a dramatic contribution to biological exploration.

APPROACH TO CURRENT STUDY

In January of 1962, the Aerospace Research Department of General Mills, Inc. undertook a program of unmanned balloon flights under contract to the National Aeronautics and Space Administration to ascertain the presence of and distribution of viable microorganisms in the stratosphere between 30,000 m and the tropopause.

The following research philosophy guided our efforts, for we were determined to overcome the criticism and limitations of prior investigations.

Assuming that microbial concentrations near ground level are significantly greater than those at higher altitudes, the major problem was to obtain a true stratospheric sample uncontaminated by material from lower strata. Therefore, the approach chosen was to send a protected payload aloft to a predetermined height, and there to initiate a sampling program during controlled descent through the profiles of interest.

Since the only previous estimate of microbial concentration in the stratosphere (Rogers & Meier, 1936) was in the range of one organism per 10 m^3, a series of samples with volumes from 300 to 3000 m^3 would be required. This sample size would conceivably yield high enough counts to make the "signal" significant compared with the background "noise" that would be provided by accidental contamination.

Accidental contamination during fabrication, storage, flight and recovery could be minimized by the following precautions:

1) The assembled samplers would be wrapped like surgical instruments and autoclaved. Once autoclaved, the interior of the samplers would remain protected until the appropriate sampling altitude was reached.

2) The exterior of the samplers would become contaminated after removal from the autoclave, during attachment to the gondola, and during storage preparatory to suitable launch conditions. But the external contamination would not have access to the interior of the samplers, and would be minimized by our treating the entire assembled payload with Ethylene Oxide during storage.

3) During launch and ascent, air- and dust-borne organisms from ground level could conceivably be contamination sources. So we devised a system of jettisonable covers on the inlet and outlet ports to protect the interior until sampling altitude was reached. The pressure differential between the interior and exterior of the sampling chambers during ascent would also tend to protect the interior.

4) During descent through the stratosphere, after the protective covers had been jettisoned and the sampling sequence had been initiated, the only serious sources of accidental contamination would be the surface of the balloon, the gondola, and the parachute, all of which might have entrained soil, dust, and organisms from ground level during preparation and launch. The significance of this contamination source would be minimized by programming the descent rate, the sampling rate, and the dimensions of the inlet port to assure isokinetic sampling. Thus, small particles from the balloon would be falling "away" from the inlet, and large particles would fall "past" the inlet. With proper design and programming, we hoped that the equipment would sweep through a narrow, vertical column of undisturbed air having the same (or smaller) diameter as the inlet cone. We also planned to take control samples of air to ascertain the "fall out" from the balloon and equipment.

5) The most serious contamination sources anticipated were the air and dust encountered during the final several hundred meters of descent, and the soil and dust that would be aerosolized upon impact. These hazards were to be eliminated by an automatic sealing gate which would snap shut immediately after the sampling sequence in the stratosphere was completed. This gate and suitable gasketing would be designed to retain integrity of the units' interior during descent below the tropopause, during impact, and during transport to the analytical laboratory.

The basic sampling process would involve large-volume air filtration. We recognized that filtration might be inimical to viability and that vegetative cells might be killed by our blasting air across them at high velocities. But, these considerations were weighed against the need of acquiring large samples with relative ease in a limited time period. It was thought that any organism already present in the stratosphere would be sufficiently hardy to withstand the potentially lethal forces imposed during filtration.

This project was an excellent example of the need for cooperation and interaction between a variety of scientific disciplines. The design and testing of the sampling equipment alone was an exercise in mechanical, aeronautical, and bioengineering. The sampler had to meet certain specifications of weight, ruggedness, reliability and automation, and yet had to conform to the needs imposed by a microbiologist concerned with the sterility, contamination, and aseptic handling of electromechanical hardware. The sampling programs had to be predetermined with regard to geography, engineering, electronic instrumentation, and meteorology. Ultimately, new laboratory techniques had to be developed whereby the filters could be aseptically removed from the samplers and analyzed. It was hoped that the initial program (involving two flights in 1962) would generate sufficient information about the biological and engineering aspects of this type of research to permit a more systematic and comprehensive exploration in 1963. This second phase is currently under way.

APPARATUS AND TECHNIQUES

Sampling Equipment

A variety of potentially suitable filter materials was tested for possible use in the sampler. Among the materials tested were membrane filters, IPC paper, polystyrene paper, Fiberglas and polyurethane foam. After consideration of filtration efficiency, chemical and biological inertness, low pressure drop, stability in simulated stratospheric environments, ease of sterilization and ease of recovery of organisms from filter matrix, polyurethane foam (80-pore size) was chosen as the filter material. Sheets of polyurethane foam could be cut to a desired size and shape to fit the sampling apparatus. After a flight, the filter could easily be dissected out of the apparatus and the organisms adhering thereto quantitatively extracted. Table II describes the efficiency of polyurethane foam against artifically generated microbial aerosols (≈ 1 μ diam.) in an altitude-simulation chamber.

Table II. Collection Efficiency of Polyurethane Filter (80 Pore)

Simulated Altitude (km)	Filter Thickness (cm)	Linear Velocity of Airflow (m/min.)	Collection Efficiency (%)
9	No filter	152	0
3	2.5	115	50
9	2.5	128	60
9	2.5	153	78
14	2.5	165	91
14	2.5	249	> 99
18	1.3	274	> 99
18	1.3	226	> 99
27	1.3	224	> 99

The sampling payload consisted of four direct-flow sampling units, mounted vertically on the four corners of a gondola, in the center of which were nested the power packs and the regulating and recording instruments. The air inlet pointed downward for sampling during descent, and the air was exhausted through a high-altitude PR-2 flowmeter attached to a recording device in the gondola. Each sampling unit was fitted with a circular filter of polyurethane foam (0.087 m^2) supported on a wire mesh. Air was pulled through the filter by a Torrington No. 704 blower powered with a Westinghouse d-c aircraft motor (27v, 24 amp, 0.52 hp, 12,300 rpm). The skin of the unit was of spun aluminum, the frame was of tubular aluminum, and the inlet cone was of sheet aluminum. Each sampler measured 1.2 m by 0.58 m in diameter and weighed approximately 23 kg. The total payload weight of the samplers, gondola, instrumentation, and batteries was approximately 300 kg. It was anticipated that upon impact the inlet cones would collapse and serve as shock absorbers. They were therefore designed to be expendable. The remainder of the unit was designed to be reusable. (It has been flown successfully in the stratosphere three times.) A schematic drawing of an individual direct-flow sampler is shown in Figure 1, and a photograph of the apparatus (without protective covers) is presented as Figure 2.

After the samplers had been assembled and the filter pad clamped into place, the inlet cone and flowmeter exhaust were fitted with gasketed aluminum dust covers. A nylon shroud was pulled over the inlet cone and secured with cord. The shrouds and dust covers were released simultaneously as the balloon approached sampling altitude by firing squibs which severed the securing cords. The covers then descended by parachute, leaving the samplers exposed. Each unit contained a spring-loaded, self-locking gate, which was cocked open during assembly and which remained open during sterilization, storage, launch, ascent and sampling (see Figure 2). At the termination of a sampling sequence, a squib was fired which released the spring on the sealing gates. The gates were gasketed with polyurethane foam and, when shut and secured, protected the filter from extraneous contamination.

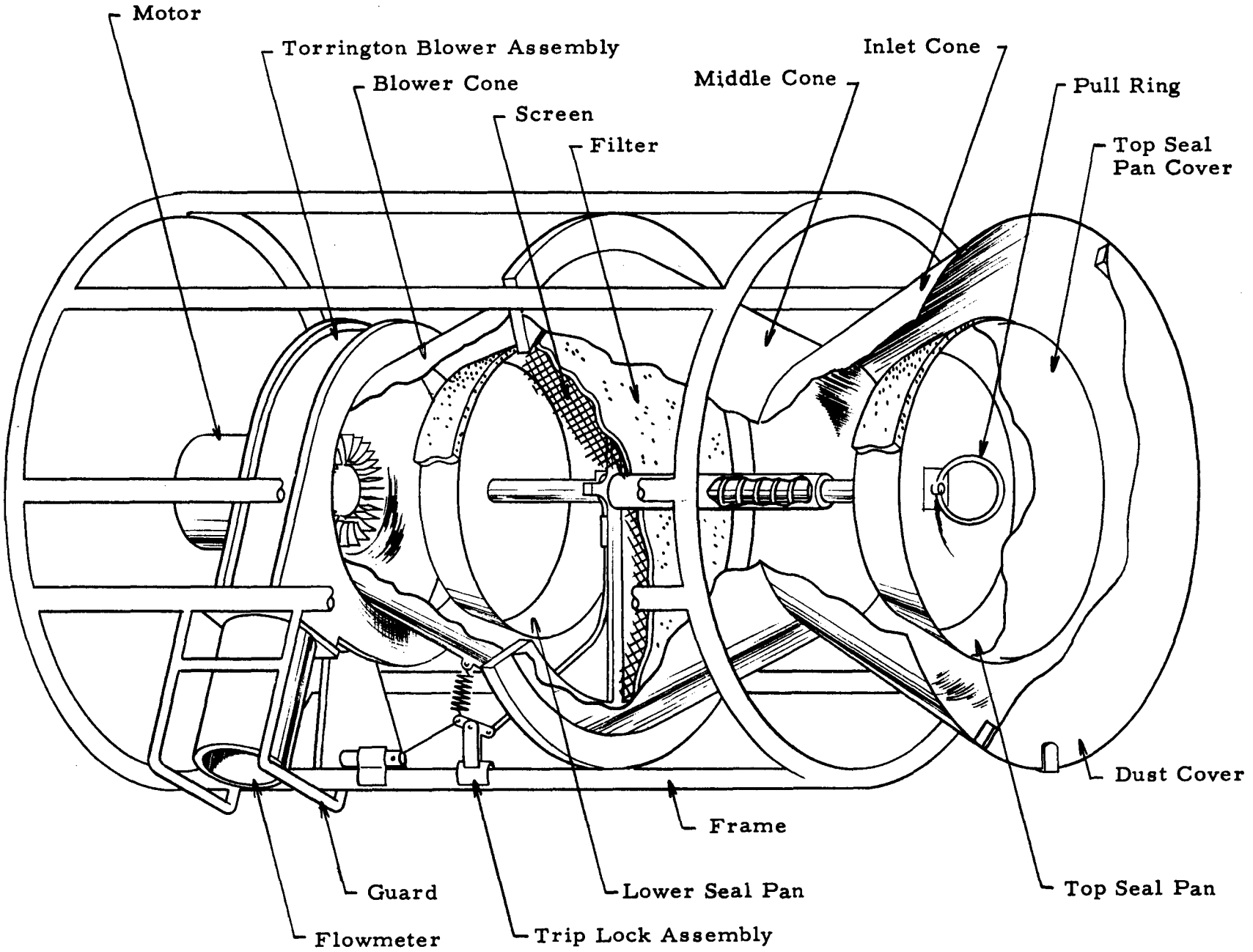
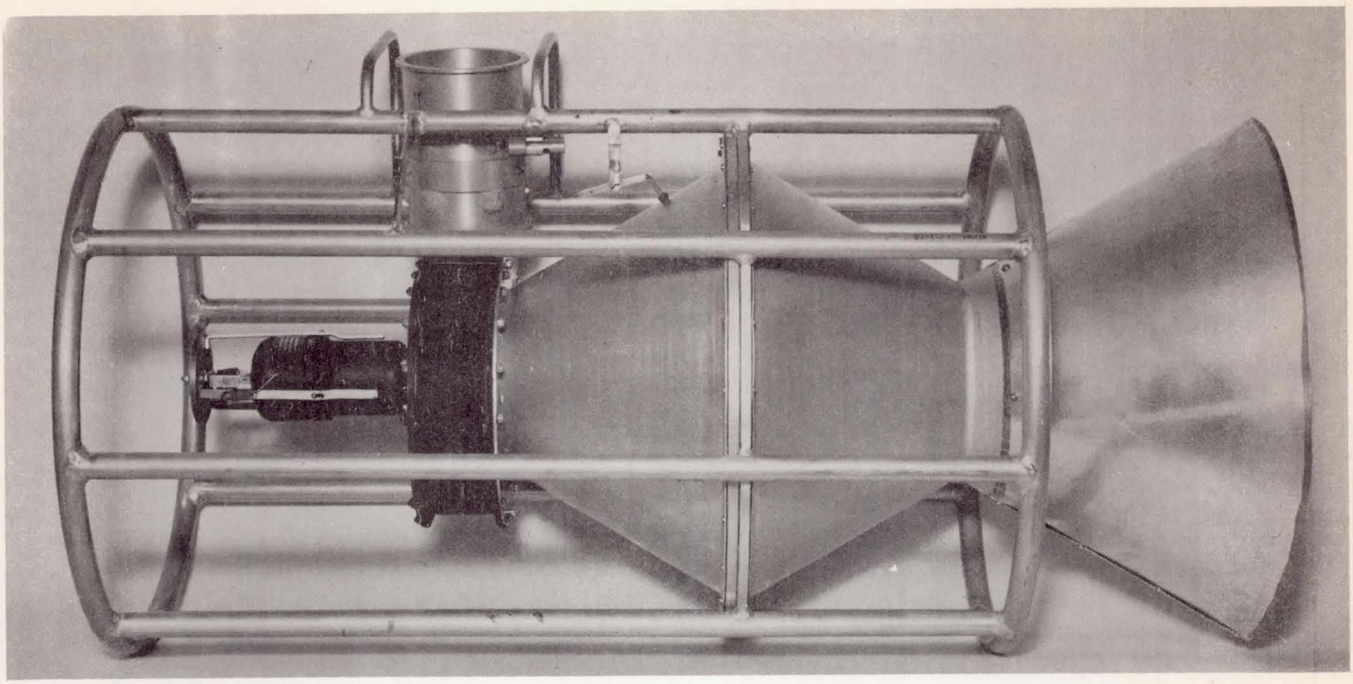
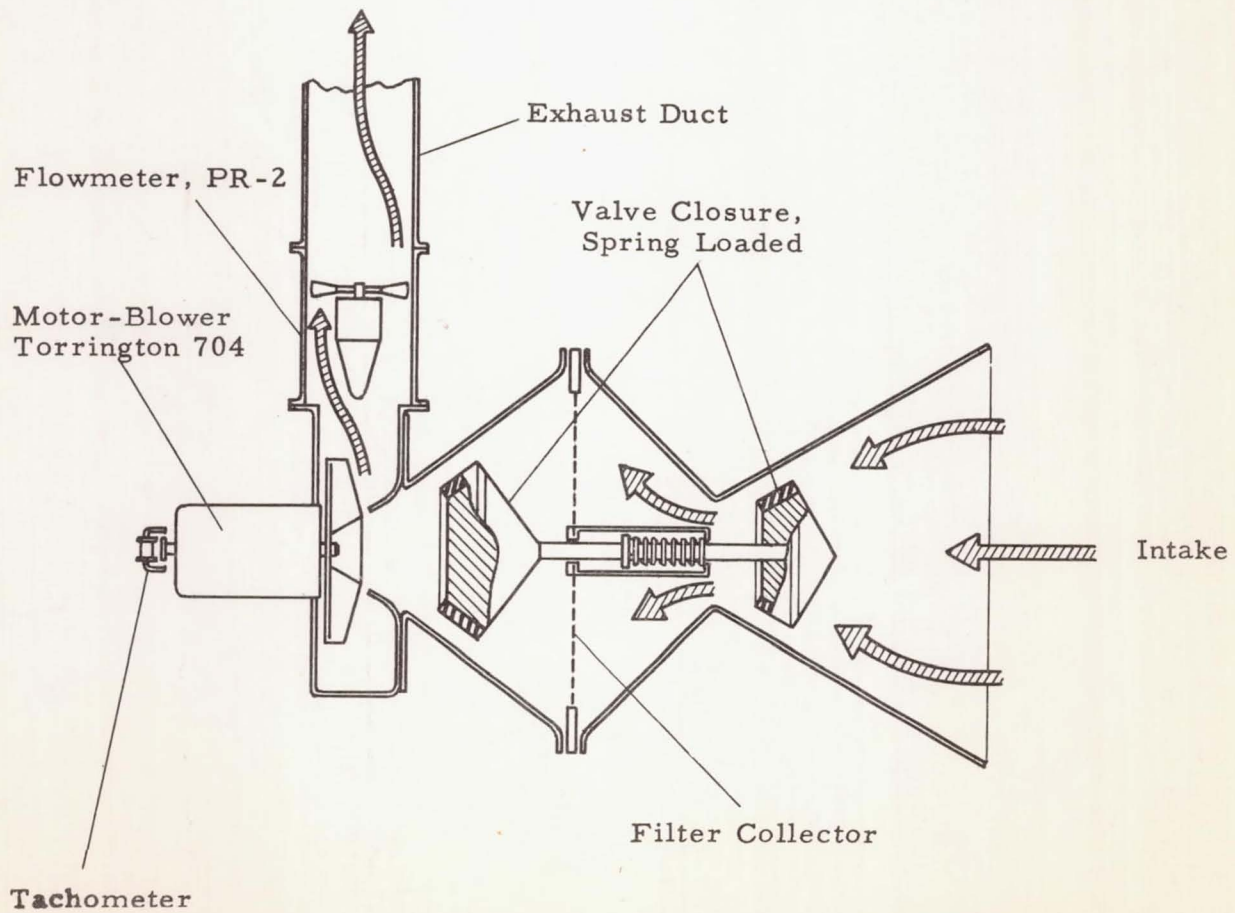


Figure 1. Schematic Drawing of Sampling Unit



2-1. Unit Ready for Attachment to Gondola



2-2. Airflow Pattern through Unit in "Cocked-Open" Position

Figure 2. Individual Sampling Unit

Before flight, the assembled and protected units were wrapped in surgical wrapping paper and sterilized in hospital autoclaves at 120 C for one hour. Still wrapped, the units were attached to the gondola, and the complete payload placed in a polyethylene shroud (see Figure 3). During storage, the atmosphere in the shroud was under ethylene oxide-freon pressure. Extensive surface sampling of the payload indicated that the exterior surfaces had less than one organism per 45 cm^2 immediately before launch. The filter pad itself was sterile. Just before launch, the paper wrappings and large polyethylene shroud were removed, leaving the dust covers and nylon shrouds protecting the sampler. The internal surfaces of the sampling units were not exposed between the time of autoclaving and the attainment of stratospheric altitudes.

Before we conducted an actual flight, several simulated "flights" were performed in an altitude chamber to verify the reliability of the automatic timers, barometric switches, blower, squibs, flowmeters, and sealing gates. Typical data from one of these simulation "flights" are presented in Table III. It was evident that the mechanical apparatus performed well after sterlization and exposure to the stratospheric environment.

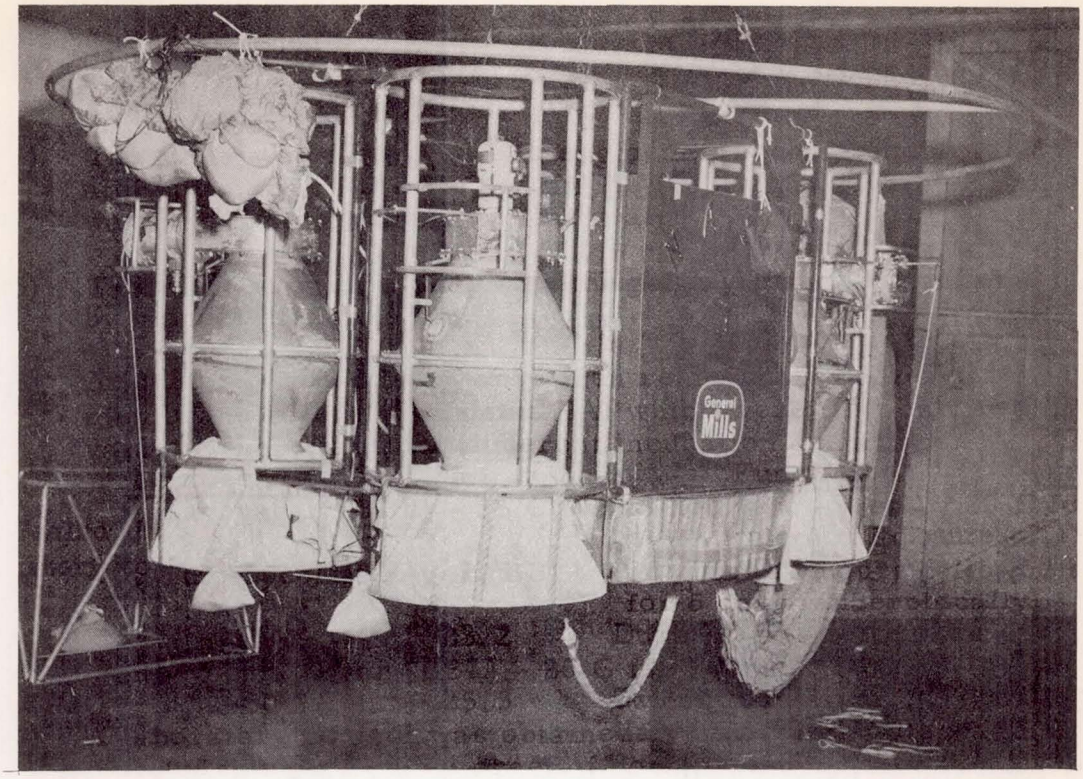
LABORATORY TECHNIQUES

The following protocal was developed for the recovery and analysis of the biological sample in the laboratory:

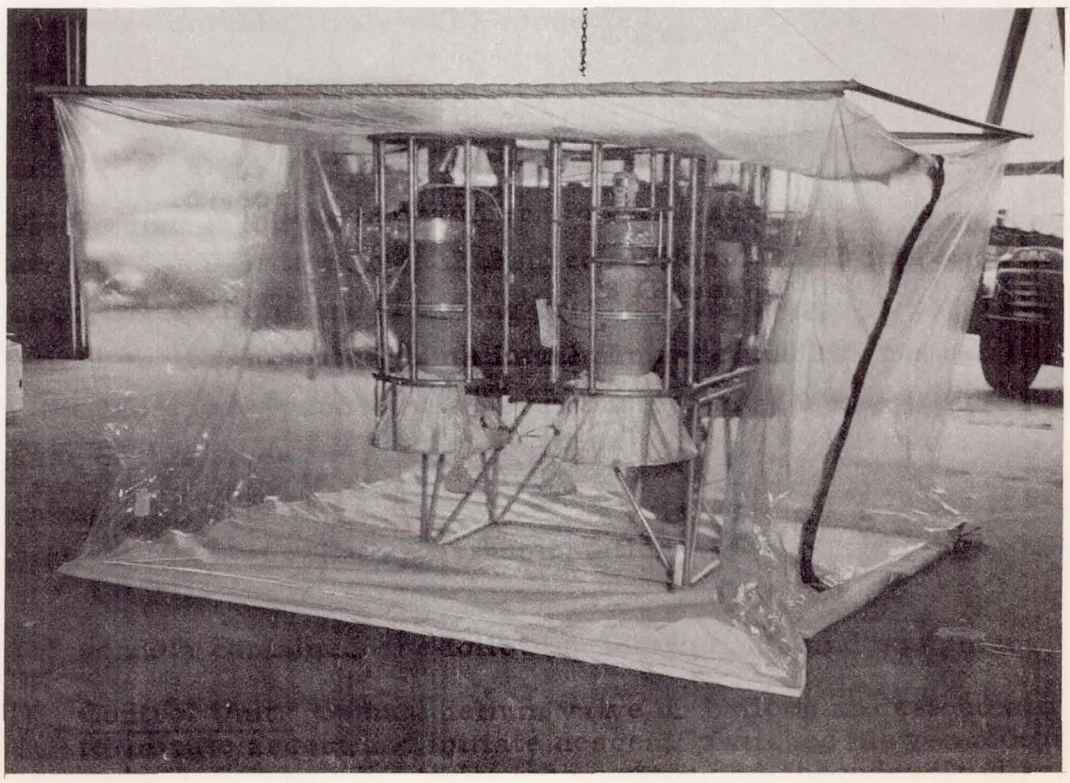
1) At impact site, the sampling units were examined for obvious leaks and malfunctions, were detached from the gondola, shrouded in clean polyethylene bags, and returned to the laboratory.

2) In the laboratory the bags were removed, and the exterior surfaces of the units were thoroughly cleaned and disinfected with a phenolic detergent-germicide.

3) The unit was aseptically disassembled in a white room, and the filters pad was exposed for the first time since completion of sampling in the stratopshere



a) After Attaching Samplers to Gondola



b) Storage in Ethylene Oxide Atmosphere

Figure 3. Final Payload

Table III. Log of a Simulated Flight in Altitude Chamber

	Temperature	Simulated Altitude km	Function	Airflow Thru Filter Ambient m^3/min.
Ascent	-50	1.5	Antenna Drop	
	-50	23.2	Dust Covers Jettisoned	
	-50	25.3	Switch to Helium Valve On	
	-50	27.4	Sampling Systems Armed	
Descent	-50	26.8	Blowers 1 & 2 Start	20.9-27.6
	-50	26.5	Blower 1 Off; Sampler 1 Closed	21.5-28.3
	-50	18.3	Blower 2 Off; Sampler 2 Closed; Blower 3 On	21.2-28.9
	-50	15.2		20.2-26.6
	-50	12.2	Blower 3 Off; Sampler 3 Closed	17.5-21.6
	+30	Ground Level	Impact Switch Fires	

4) The filter was dissected into segments: each segment was immediately placed in a sterile polyamide (capran) bag with 100 ml of sterile water; the bags were then heat sealed.

5) The filter was repeatedly and thoroughly extracted with the diluent by manual manipulation.

6) Aliquotes of the diluent were then removed and filtered through membrane filters (Millipore HA) which were then cultured on a variety of media under different incubation conditions:

Eugonagar (BBL) - 35 C for 48 hours followed by 20 C for 5 days

Thioglycollate Agar (BBL) - 35 C for 48 hours followed by 20 C for 5 days (anaerobically)

Mycophil Agar (BBL) - 20 C for 7 days

7) A laboratory control was obtained by performing steps 4), 5), and 6) on a freshly autoclaved sheet of polyeurethane foam.

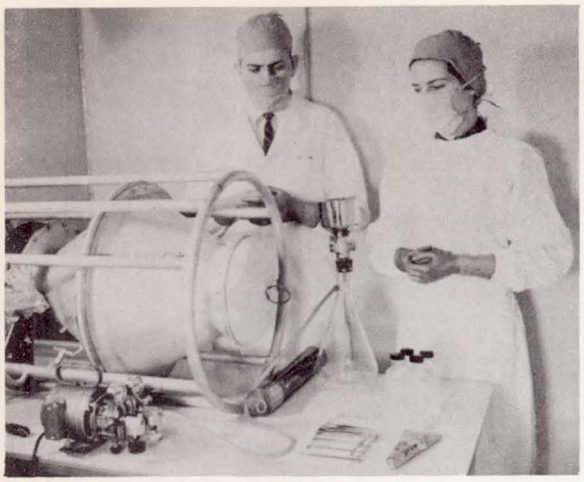
The sequence of activities performed in the laboratory is illustrated in Figure 4.

Many preliminary trials were conducted to ascertain the practicality of these techniques and to measure both the incidental contamination inherent in the method and the efficiency of microbial recovery from artificially contaminated filters. A summary of these preliminary trials is given in Table IV. Further trials in an altitude chamber, in which aerosol counts upstream and downstream from a filter were measured by liquid impingers showed that the collection, extraction, and culturing procedures chosen yielded 35-75% of the theoretical numbers of organisms in the air. Because of the success of these tests, the laboratory procedures were considered adequate for the bacteriological phase of this study.

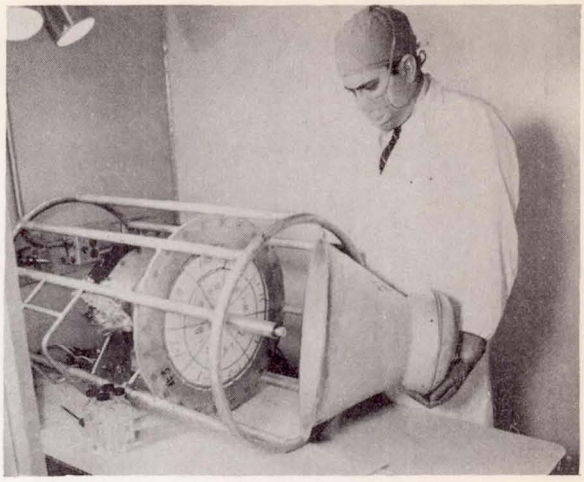
Instrumentation

The gondola contained the following instruments and equipment:

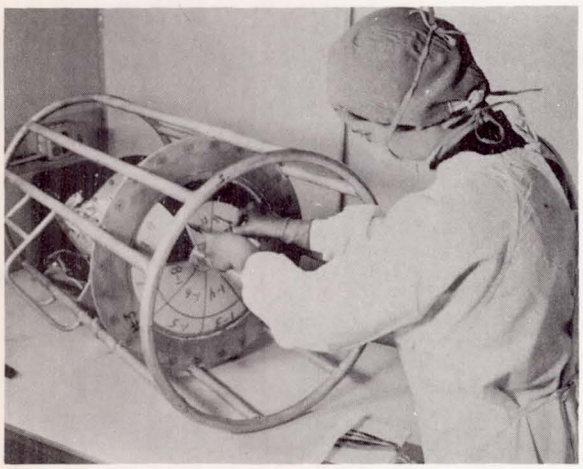
1) Control Unit: Opened helium valve on balloon at desired altitude to terminate ascent and initiate descent; switched blower motors on and off; fired squibs to jettison covers and shrouds; fired squibs to release spring-loaded sealing gates.



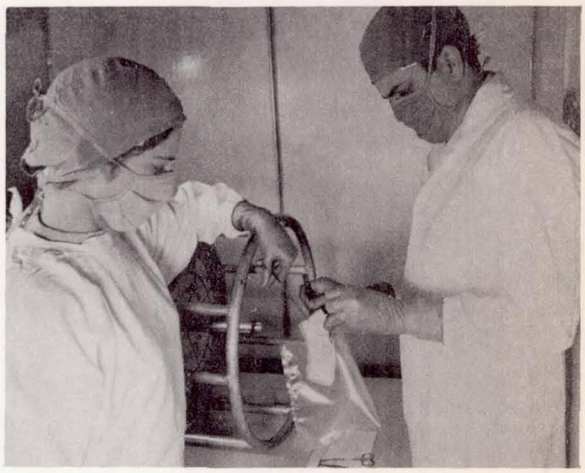
-1. Sealed Unit in White Room Preparatory to Filter Exposure



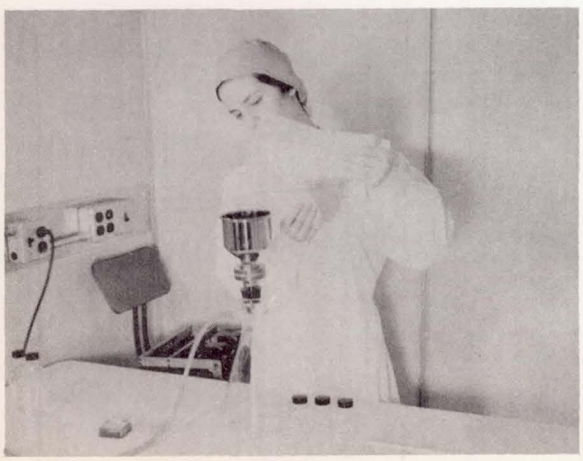
-2. Aseptic Exposure of Filter Pad



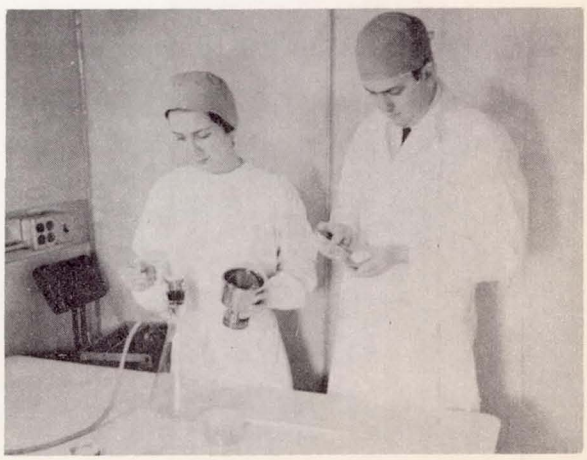
-3. Dissection of Filter Material from Frame



-4. Bagging of Filter Segment



-5. Extraction and Membrane Filtration



-6. Plating Membrane Filters

Figure 4. Laboratory Sequence of Filter Analysis

Table IV. Incidental Contamination Contributed by Analytical Technique and Recovery Efficiency of Artificial Contaminants

Trial	Inoculum Size	Recovery after Extraction 1st	2nd	3rd	4th	Total Recovery	Sterile Control Total Count Per 1000 cm^2
1	8,800	7,600	280	14	5	7,899	9
2	880	330	11	0	0	341	16
3	88	69	4	0	0	73	8
4	6	4	0	0	0	4	20
5	9,700	7,400	860	-	-	8,260	5
6	970	840	91	10	-	941	10
7	21,000	16,000	4,600	-	-	20,600	1
8	21,000	22,000	-	-	-	22,000	1
9	21,000	20,700	-	-	-	20,700	1
10	21,000	22,500	-	-	-	22,500	1

2) Barocoder and 5-Watt Transmitter: Telemetered altitude; used as homing station for tracking aircraft; indicated functioning of blowers.

3) Flowmeter Recording Unit: Measured meter revolutions, inlet and exhaust air temperatures, and pressure drop across filters; recorded information on synchronized film.

4) Tilt Switch: Released and destroyed the balloon upon impact to prevent damage to payload by dragging.

5) Power Supply: Main power supply was 28v dc with a capacity of 135 amp-hr.

The flights were monitored from a ground control station, and a record of the functions was obtained by a camera mounted in the instrument pack. Tracking aircraft spotted the impact zone and directed recovery vehicles to the site by radio.

EXPERIMENTAL RESULTS

Flight Descriptions and Data

During 1962 two flights were made: to 19.8 km on August 1, and to 27.1 km on October 19. The same basic flight train (Figure 5) was employed each time, the major differences being size of the balloon and programming of the samplers.

As we progressed in this work, we learned much about the possibilities and limitations of this type of exploration. Consequently we experimented with different program sequences in order to improve the reliability of whatever data we obtained. Some minor modifications are presently being incorporated into the program of four flights currently underway. Table V summarizes the flight data from the 1962 probes.

During Flight No. 1, all units ascended in the "cocked-open" position, protected by dust covers, which were jettisioned at 3 km. The balloon reached float altitude at 19.8 km. The Sampler 1 blower turned on when descent started and sampled a profile of air from 19.8 to 13.7 km, where its blower turned off and its sealing gates closed. Sampler 2 served as a

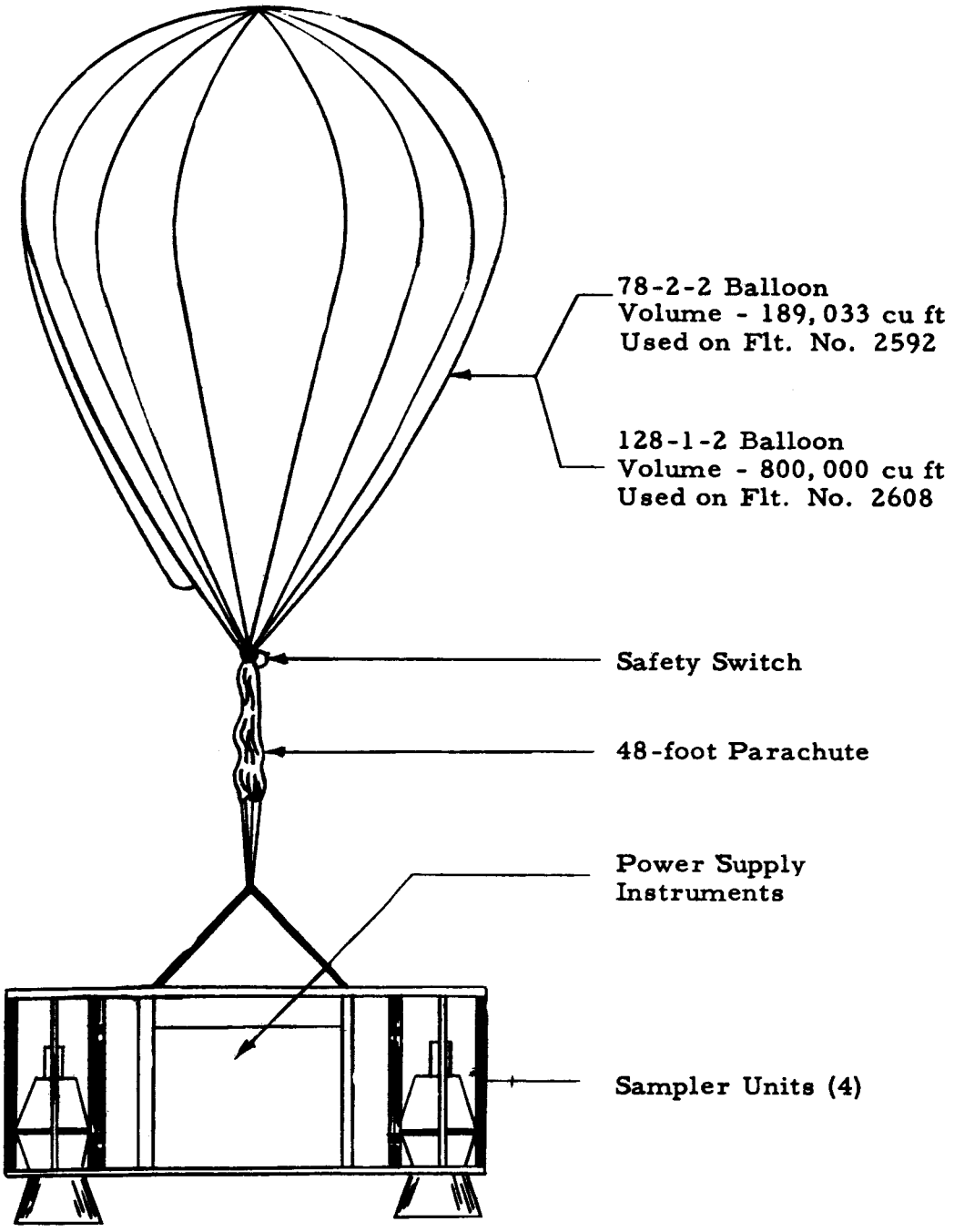


Figure 5. Flight Train

Table V. Summary of Flight Data

	Flight 1	Flight 2
Date	August 1, 1962	October 19, 1962
Balloon Description	23.8 m diam; 5,384 m^3; 2-mil polyethylene	39 m diam; 22,792 m^3; 2-mil polyethylene
Payload	268 kg	293 kg
Launch Procedure	truck	two balloon
Time of Launch	0548 CST	0739 CST
Rate of Climb	320 mpm to 19.8 km	285 mpm to 26.8 km
Dust Covers Jettisoned	3.0 km	24.4 km
Floating Time	50 min	40 min
Rate of Descent	114 mpm (19.3 km to 13.7 km)	33.5 mpm (27 km to 24.4 km)
	269 mpm (13.7 km to 9.1 km)	112.8 mpm (24.4 km to 18.3 km)
	305 mpm (9.1 km to 3.0 km)	217 mpm (18.3 km to 12.2 km)
Impact Area	oat field (Fountain City, Wisconsin)	forest (Lily, Wisconsin)
Condition of Impact	gates on all units except 2 shut and locked; gates on unit 2 shut, not locked	gates on 1 and 2 shut, not locked; gates on 3 shut and locked; gates on 4 not shut tight
Arrival at Laboratory	1600 CST	0030 CST (October 20)

control to Sampler 1. It descended open through the same profile and its gates closed at the same altitude, but its blower remained off. The contamination recovered from it would consequently be accounted for by storage, ascent, impact, and whatever it entrained by ram impaction in the stratosphere. Sampler 3 sampled a profile in the tropopause from 13.7 km to 9.1 km. Sampler 4 operated between 9.1 km and 3.0 km.

During flight No. 2, Samplers 1, 2, and 3 ascended in the "cocked-open" position protected by dust covers until 24.4 km. Samplers 1 and 2 both switched on after descent started at 27.1 km. No. 2 sampled about 100 m^3, and shut off and closed its gates after 3 minutes. It served as a "float control" to measure contamination which originated from the balloon and payload. Sampler 1 continued to sample during descent through a profile between 27.1 km and 18.3 km. Sampler 3 operated between 18.3 km and 12.2 km. Sampler 4 was treated in an identical fashion as the other three until just prior to launch. At that time its sealing mechanism was manually activated. It ascended and descended closed, and was designed to measure incidental contamination during storage, from leakage in the stratosphere, and from impact. Photographs of one launch are shown in Figure 6. Sampling data from the recording instruments are summarized in Table VI.

Microbiological Data

Although the first two flights were technologically successful, the microbiological results still do not permit us to draw any unequivocal conclusions about the existence of viable organisms above the tropopause. Several types of bacteria and fungi were isolated from filters exposed in the stratosphere, and from one sampler (Flight 1, Sampler 1, 19.7 km to 13.7 km) a surprisingly large count was obtained. However, since the "control" samplers also showed contamination, and since the results from the two flights were quantitatively and qualitatively different, the data must be subjected to careful analyses before any attempt to draw dramatic conclusions.

The microbiological data are summarized in Table VII.

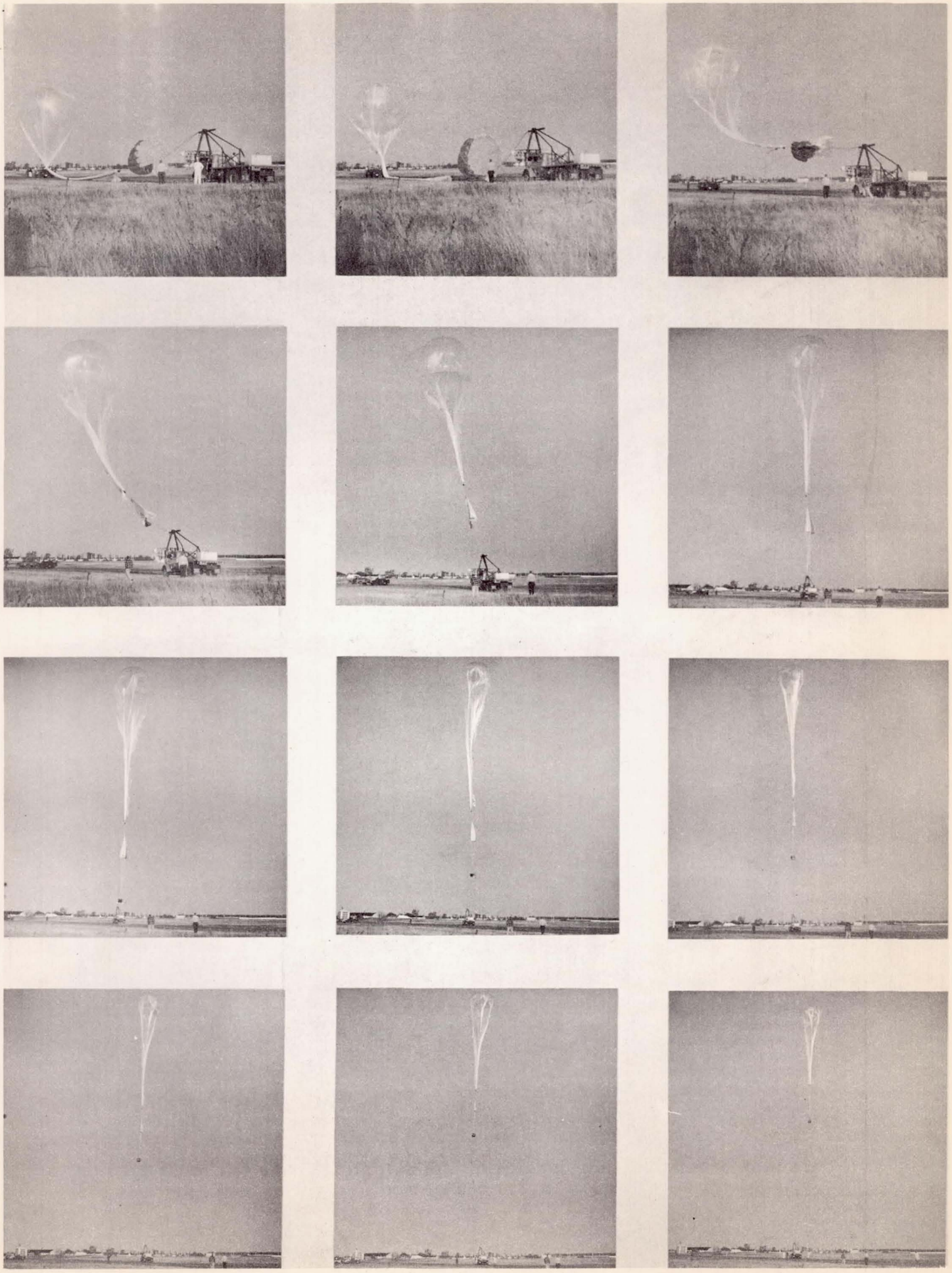


Figure 6. Launch Sequence

Table VI. Sampling and Environmental Data

		Filter Thickness (cm)	Profile Sampled (altitude in km)	Sampling Time (min)	Ambient Temperature (°C)	Ambient Flow Rate (m^3/min)	Volume Sampled	
							Ambient (m^3)	STP (m^3)
Flight 1	Unit #1	1.27	19.7 to 13.7	54	-58 to -66	22.9-29.7	1700	148
	#2	1.27	Flight Control					
	#3	2.54	13.7 to 9.1	16	-60 to -66	10.2-15.6	249	55
	#4	2.54	9.1 to 3.0	20	-37 to -45	7.6-10.5	170	83
Flight 2	Unit #1	1.27	27.0 to 18.3	156	-34 to -50	24.3-26.3	3976	142
	#2	1.27	27.0 (float control)	3	-56	27.4	113	2
	#3	1.27	18.3 to 12.2	22	-41 to -55	26.8	578	75
	#4	1.27	Impact Control					

Table VII. Summary of Bacteriological Results

Flight & Sampler No.	Volume of Ambient Air Sampled (m^3)	Microbial Count Recovered		Predominant Organism
		Molds	Bacteria and Yeasts	
August 1, 1962				
1 19.7 km to 13.7 km	1700	8000	13,000	Yeasts Flavobacterium sp Brevibacterium sp Corynebacterium sp Alternaria sp Cladosporium sp
2 Flight Control	--	64	240	Yeasts Brevibacterium sp
3 13.7 km to 9.1 km	249	370	120	Penicillium sp
4 9.1 km to 3.0 km	170	56	56	Penicillium sp
October 19, 1962				
1 27.0 km to 18.3 km	3976	22	106	Micrococci Aspergillus sp
2 27.0 km (Float Control)	113	15	45	Micrococci
3 18.3 km to 12.2 km	578	56	140	Micrococci Cladosporium sp Aspergillus sp
4 Control	--	46	234	Actinomycetes Micrococci Aspergillus sp Cladosporium sp

After the first flight, a considerable quantitative difference was evident between the samplers. Laboratory controls indicated that relatively few organisms were contributed by our techniques. The flight control (Sampler 2) suggested that some extraneous contamination had occurred, but since this unit had partially malfuntioned upon impact and since the gates were not locked, the counts may possibly reflect the malfunction. The extremely high count in Sampler 1 was surprising. The counts from Samplers 3 and 4 were not significantly different from the control.

Qualitative differences between the samplers were evident from gross observation of the culture plates. Subsequent isolation and detailed characterization procedures revealed that the filters exposed at different altitudes contained different predominating flora. The filter from Sampler 1 contained about 20,000 organisms, the majority of which were members of the pigmented genera Flavobacterium sp, Brevibacterium sp, and Corynebacterium sp. From this filter we also isolated a large number of white nonfermenting yeasts, some Rhodotorula sp, several thousand Alternaria sp, and Cladosporium sp. Although we searched carefully we could find no spore-forming bacilli, no actinomycetes, and no Aspergilli or Pencillia in this sampler. The flight control contained the same types of organisms as Sampler 1, although the total count on this filter was two orders of magnitude lower. The predominant organisms on the filters exposed below 13.7 km (Samplers 3 and 4) were Penicillium sp. The few bacteria encountered were similar to those on Sampler 1.

The results from the second flight were considerably different. None of the filters yielded counts close to those observed from the first flight. We were encouraged by the very low count in the float control sample, which suggested the low "noise" level attributable to balloon fall off. Even the relatively high count from what was supposed to be a sterile control (Sampler 4) was acceptable, considering that the sealing gates on this sampler sprung open after the payload knocked down two trees during impact. We do not, however, feel that the two-fold difference between the counts from Samplers 1 and 3, or 3 and 2 is sufficient to ascribe significance to the data from the second flight. Furthermore, the qualitative data also

suggest the possibility of accidental contamination. The predominance of Cladosporium in Sampler 3 was reminiscent of the first flight. In general, the types of organisms isolated from these filters were too similar to the common variety of forest air and dust flora which we sampled at the impact site.

On the basis of the results from the first two flights, we are satisfied that the apparatus and general exploration approach works well. We are not yet ready to make any unqualified statements about stratospheric microbiology until we have performed further experiments.

DISCUSSION

Two basic problems must be resolved when interpreting the data from this program:

1) Are the results valid?

2) How can the discrepancies between the flights be resolved?

Arguments designed to support the validity of these findings are based on the following considerations:

1) Preliminary experiments demonstrated the validity of the sampling and analytical techniques.

2) Viable organisms differing both quantitatively and qualitatively were isolated from the different filters, which had been exposed to moving airstreams at different altitudes.

3) For a given flight, the higher counts were generally found on those filters through which large volumes of ambient air had been sampled.

4) Common soil and dust organisms normally encountered in the environment were not isolated from the filters exposed during the first flight, whereas they were found in a sampler which obviously malfunctioned during impact at the end of the second flight.

5) The limited variety of types isolated after the first flight, the large numbers found in one sampler compared with its control, and the uniform distribution of organisms on the filter diminish the probability that this was all extraneous contamination.

Arguments designed to deny the validity of these findings are based on the following considerations:

1) There are many complex and serious sources of accidental, low-level contamination which might account for the observed results.

2) All of the organisms isolated are common inhabitants of the oat field in which the first payload impacted.

3) A 268 kg payload impacting at 305 meters per minute could conceivably aerosolize a large cloud of dust, which in turn might account for the large numbers of organisms found in a given sampler.

4) Although the sealing gates might be locked, the impact could have interfered with the sealing integrity sufficiently to permit contamination.

5) The controls were not sterile.

It is hardly necessary to point out that both points of view are scientifically valid, and that even with extensive speculation the argument can not be resolved simply by the results of these first two probes. In the current continuation of this program, further attempts are being made to improve the reliability of results. The balloon is being dusted with particles of fluorescent zinc cadmium sulphide, and the filters will be examined for the presence of these particles after a flight. Some mechanical improvements are being made in the gasketing and locking mechanisms. Extensive environmental samples are being taken at both launch and impact sites. We hope that the next few flights will provide us with a payload in which all the samplers function well and lock after sampling, and in which the controls are sterile.

Whether or not microorganisms exist in the stratosphere, it must be recognized that meteorological mechanisms do exist for the introduction of terrestial particles into that environment. Furthermore, the conditions of low temperature, low oxygen tension, low humidity and low air pressure are compatible with microbial survival for long time periods. It is entirely possible that microorganisms might be encountered at the altitudes which were sampled, though we are not completely convinced that the cultures we obtained do in fact represent stratospheric microflora. It is tempting to point out that previous workers who sampled at lower altitudes also consistently reported the isolation of pigmented bacteria and yeasts, Cladosporium and Alternaria.

The reconciliation of differences in the results of the two flights is similar to that of reconciling differences between two 1-milliliter samples of the Pacific Ocean taken two months apart from two sampling locations. Conceivably, two probes launched simultaneously and programmed to sample identical air masses would yield discrepancies. Since, in fact, the two probes reported were launched eleven weeks apart, disagreement should be expected from probability considerations alone.

However, the most significant sources of variation between the two flights were the meteorological parameters. An analysis of the air trajectories at different altitudes in the geographical zone of our flights revealed the following: On August 1, air in the 14-20 km profile was flowing from the WNW, originating in Alaska 5-6 days previously. Immediately above 20 km there was a flow from the east, originating over the Atlantic 4 days previously. On October 19, the prevailing air throughout the 12-27 km came from Alaska, flowing from the west. It is entirely possible that the discrepancies in the bacteriological results might be caused by the differences in the origins of the stratospheric air masses during the probes.

POSTSCRIPT

On May 11, the third flight in this series was successfully launched and recovered. We attained the same altitude as that of Flight No. 2 and are in the process, at time of writing, of analyzing the samples. On the basis of very premature and fragmentary results, we made the following preliminary observations:

1) Fluorescent particle contamination from the balloon was insignificant.

2) The sterile control functioned well; the count will be less than 10 organisms per filter pad.

3) Most of the filter pads were contaminated with the same type of organisms that were found in great numbers in the dust and on the impact area, indicating a probable malfunction of the sealing mechanisms.

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*Contain bibliographies of authors listed in Table I, pp. 3-5.

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